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The effect of activated charcoal and auxins on root formation by hypocotyl segments of *Daucus carota*

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The adsorption of 2,4-dichlorophenoxyacetic acid (2,4-D) by activated charcoal (AC) from methanol and aqueous solutions was determinated using HPLC. The level of the added 2,4-D decreased in both methanol and aqueous solutions in the presence of 1% AC. About 68% and 61% of the added 2,4-D was adsorbed respectively by AC (1.0%) from these two sources. *In vitro* rooting of hypocotyl segments of *Daucus carota* using AC and the auxins 2,4-D, α-naphthaleneacetic acid (NAA) and indole-3-acetic acid (IAA) was investigated. Rooting occurred when 7-day-old seedling hypocotyl segments were placed on Murashige and Skoog medium supplemented with 1.0mgl^{-1} 2,4-D in the presence of AC, and 0.5mgl^{-1} NAA or IAA. Hypocotyl segments did not produce roots on the 2,4-D-containing medium in the absence of AC. Hypocotyl segments produced roots polarly on the NAA or IAA-containing media in the presence of activated charcoal. No-polarity of root formation was observed on media supplemented with NAA or IAA without AC. Different responses of hypocotyl segments to various levels of 2,4-D (0mgl^{-1}, 0.5mgl^{-1}, 1.0mgl^{-1}, 3.0mgl^{-1}, 5.0mgl^{-1}, 8.0mgl^{-1} and 10.0mgl^{-1}) were observed on media supplemented with 0.02%, 0.1% and 0.5% AC. Root number per hypocotyl segment decreased in the NAA or IAA-containing media in the presence of AC. However, root number per hypocotyl segment, on the media supplemented with NAA or IAA, increased when the segments were pre-cultured on MS medium supplemented with 2,4-D (1.0mgl^{-1}) for 2–3 days. When hypocotyl segments were pre-cultured on a 2,4-D-containing MS medium for 5 days, embryos emerged from the segments directly on the medium supplemented with AC.

Abbreviations: AC = activated charcoal; 2,4-D = 2,4-dichlorophenoxy acetic acid; MS = Murashige and Skoog (1962) salts and vitamins; IAA = indole-3-acetic acid; NAA = α-naphthaleneacetic acid; IBA = indole butyric acid

Introduction

Activated charcoal (AC) is characterised by a high adsorptive capacity for gases, vapors and colloidal solids and is produced by destructive distillation of woods, peat, nut shells, bones, vegetables or other carbonaceous matter. The properties of AC are attributed mainly to its highly porous structure and relatively large surface area. The adsorptive capacity of AC generally is dependent on a variety of factors such as density, purity and pH (Halhouli *et al.* 1995).

Auxin is an essential factor for root growth. It is very widely used in micropropagation work and is incorporated into nutrient media to promote the growth of callus, cell suspensions or organs, and to regulate morphogenesis. The effects of auxins are generally not absolute or specific. The responses of cells, tissues and organs *in vitro* can vary according to culture conditions, the type of explant and the plant genotype. Activated charcoal used in nutrient media has an adsorption preference for moderately polar rather than apolar or highly polar organics. They show greater adsorption for aromatic than olefinic unsaturation products (Yam *et al.* 1990). Therefore, aromatic compounds such as the phenolics and their oxidates, auxins (2,4-D, IAA, NAA, IBA) and cytokinins [benzyladenine (BA)], could have adsorption affinity for AC. Growth and morphogenesis *in vitro* are regulated by the interaction and balance between the auxins supplied in the medium, and the growth substances produced endogenously. In contrast, the highly polar and readily water-soluble carbohydrates (glucose, sorbitol, mannitol and inositol) might not be removed from the medium and/or solution (Pan and Van Staden 1998).

Unlike IAA, NAA and IBA, 2,4-D is rarely used for root induction. There are however, reports of 2,4-D being used for this purpose. Boyes and Sink (1981) found that adventitious root formation on shoots in *Salpiglossis sinuata* was more effectively induced with 2,4-D than NAA. *In vitro* rooting on shoots of *Leptospermum flavescens* was obtained by placing shoots on a 2,4-D-containing medium (Shipton and Jackes 1986). However, 2,4-D did not induce any roots on shoots of *Anigozanthos fulginosa* (Sriskandarajah and

Mullins 1981). At concentrations less than 10µM 2,4-D inhibited rooting of *Malus x domestica*, causing both shoots and medium to discolour within a few days (Sriskandarajah and Mullins 1981).

Activated charcoal is able to adsorb substances presumed deleterious and/or inhibitory to *in vitro* culture. However, adsorption of growth regulators applied to the tissue by AC could also occur at the same time, and non-selective adsorption may result in negative effects on cultured explants. Fridborg and Eriksson (1975) suggested that AC removed growth regulators, particularly auxins, from the medium. In tissue culture media 0.1% AC can effectively adsorb 10µM IAA (1.75mgl^{-1}) and 10µM IBA (2.03mgl^{-1}) from the liquid medium (Nissen and Sutter 1990).

The experiments reported in this paper were to investigate the effect of AC and auxin interactions (2,4-D, NAA and IAA) on root formation on hypocotyl segments of carrot.

Materials and Methods

Plant material and culture media

Seeds of *Daucus carota* L. Cape Market were decontaminated in 70% (v/v) ethanol for 2min, followed by 30min in 1.05% (v/v) sodium hypochlorite and rinsed three times with sterile distilled water. The seeds were then germinated in sterile Petri dishes containing moist filter paper. Seven-day-old seedlings were subsequently again surface-decontaminated with 1.05% sodium hypochlorite solution for 20min and then rinsed five times with sterile distilled water. Ten-mm-long segments (one per seedling) were cut from the seedling hypocotyls and each of the segments placed horizontally on a medium which included MS salts and vitamins (hereafter referred to as MS medium) (Murashige and Skoog 1962), supplemented with 30gl^{-1} sucrose, 8.0gl^{-1} agar, different levels of AC and 1.0mgl^{-1} 2,4-D or 0.5mgl^{-1} IAA or NAA (unless otherwise indicated).

HPLC determination of 2,4-D

Determination of 2,4-D was achieved by reversed phase high performance liquid chromatography (HPLC). The column used was a Hypersil 25 x 0.4cm ODS C18, 5µm particle size with a flow rate of 1.0mlmin^{-1} maintained by a 3 500 p.s. single piston reciprocating pump. Absorbance was recorded with a Varian variable wavelength monitor at 280nm which was fitted with a 8µl flow through cell. Separation was achieved using a Varian 5 000 Liquid Chromatograph and the data recorded using a Vista 4 000 data system. Authentic 2,4-D (BDH) was dissolved in HPLC grade methanol (BDH). Samples were prepared by adding 0.5mgml^{-1} of 2,4-D (BDH) to both methanol and HPLC water (BDH) in the presence of 0.01%, 0.1% and 1.0% AC (BDH or Sigma). The samples were dried under nitrogen and then re-dissolved in HPLC methanol. The standard and samples were filtered with 0.2µm Millipore filters (Millipore Corporation) whereafter 10–80µl aliquots were injected into the HPLC instrument. The aqueous buffer consisted of 0.2M acetic acid adjusted to pH 3.5 with triethylamine. A linear gradient of methanol:aqueous buffer (5:95 to 95:5 over 30min at a flow rate of 1mlmin^{-1}) was used for separation. 2,4-D in the samples were tentatively identified and analysed on the basis of co-chromatography with an authentic sample. Samples were taken after 24h.

Activated charcoal treatments and culture conditions

Various concentrations of AC (Sigma) (0.01%, 0.05%, 0.1%, 0.5%, 1.0% and 3.0%) were added to the MS media before adjusting the pH to 5.8. Combinations of different concentrations of 2,4-D (0mgl^{-1}, 0.5mgl^{-1}, 1.0mgl^{-1}, 3.0mgl^{-1}, 5.0mgl^{-1}, 8.0mgl^{-1}, and 10.0mgl^{-1}) and AC (0.02%, 0.1%, and 0.5%) were added to the MS medium. For AC experiments studying the removal of compounds, 0.5% AC was added to the MS media supplemented with 2,4-D (1.0mgl^{-1}) or without 2,4-D. After the media were shaken at room temperature for 4h, the AC was removed by filtration. All media were adjusted to pH 5.8 with KOH or HCl prior to the addition of agar and after addition of AC, and autoclaved for 20min at 121°C (118kPa). All explants were incubated at 25±1°C with 16:8h/light:dark photoperiod under photosynthetically active radiation of 50µmolm^{2}s^{-1} provided by cool-white fluorescent tubes. A minimum of 20 replicates were maintained for each treatment and the number of roots per explants were recorded after four weeks of culture. The experiment was repeated twice.

Results

Effect of different levels of activated charcoal on the adsorption of 2,4-D from methanol and aqueous solutions

The levels of AC used in experiments reported in the literature vary from 0.2% to 3.0% (Pierik 1987, Ebert and Taylor 1990). It is not clear, how critical these levels are and how little may be used without exceeding its capacity to adsorb 2,4-D from the medium. To examine the capacity of AC to adsorb the standard concentrations of 2,4-D, two types of AC (Sigma and BDH) and four different concentrations of AC (0.01%, 0.1%, 1.0% and 3.0%), were added to the methanol or aqueous solutions which were supplemented with 0.5mgml^{-1} or 1.0mgml^{-1} 2,4-D. Samples of methanol and aqueous solutions were taken on the first, third, fifth and seventh day after preparation. The changes of the added 2,4-D in both methanol and aqueous solutions after one day in the presence of AC are shown in Figure 1. The results shown in Figure 1 indicate that AC adsorbs 2,4-D at all the levels used. The level of added 2,4-D decreased in both methanol and aqueous solutions in the presence of AC (Sigma and BDH). Figure 1A shows that the 2,4-D (0.5mgml^{-1}) adsorbed from the methanol solution by adding 0.01% AC (Sigma) was approximately 34.8%, while it was 31.7% in the aqueous solution when adding 0.01% AC (Sigma). About 29.5% of the added 2,4-D was adsorbed by adding 0.01% AC (BDH) in methanol solution and about 26.7% of the added 2,4-D was adsorbed by 0.01% AC (BDH) in aqueous solution (Figure 1B). Activated charcoal (Sigma and BDH) used at the level of 1.0% resulted in significantly reducing the added 2,4-D (0.5mgml^{-1} and 1.0mgml^{-1}) in both methanol and aqueous solutions (Figure 1A, B, C, D).

The changes of the added 2,4-D in methanol and aqueous

solutions over seven days in the presence of AC are shown in Figure 2. The results show that AC adsorbs 2,4-D rapidly at all the levels used. The adsorption process took place during the first day with about 28% to 68% of the added 2,4-D adsorbed depending on the type of AC and solution. On the fifth day the level of AC normally had established an equilibrium with 34% to 70% of the 2,4-D adsorbed by AC (Figure 2). The capacity of adsorption of 2,4-D did differ between Sigma and BDH AC. This was, however, not statistically significant (Figure 2). There was no significant difference in the level of 2,4-D adsorbed from methanol and aqueous solutions (Figure 2).

Hypocotyl segment growth

The hypocotyl segments cultured on the medium supplemented with 1.0mgl^{-1} 2,4-D in the absence of AC produced callus (Figure 3A), while rooting resulted from the hypocotyl segments cultured on the 2,4-D containing medium in the presence of AC (Figure 3B), and 0.5mgl^{-1} IAA or NAA-containing media in the presence or absence of AC (Figure 4). Roots generally emerged after explants were cultured on media for 4 to 6 days. Roots were observed on explants with media which contained combinations of auxins (1.0mgl^{-1} 2,4-D and 0.5mgl^{-1} IAA or NAA) and different concentrations (0.01%, 0.05%, 0.1%, 0.5%, 1.0% and 3.0%) of AC. Roots appeared earlier on the 2,4-D-containing media to which were added 0.01% to 0.5% AC rather than those containing 1.0% and 3.0% AC. The number of roots per explant decreased with increased AC concentrations on 2,4-D and IAA or NAA-containing media (Table 1, Figure 5). Addition of 0.01% AC to the medium supplemented with 1.0mgl^{-1} 2,4-D resulted in a significant increase in the number of roots produced per explant (Figure 3 and Figure 5). Rooting percentage of explants decreased on the IAA or NAA-containing media when increasing concentrations of AC were used (Table 1). However, there was no significant change in rooting percentage of explants on the 2,4-D-containing medium with increasing concentrations of AC used (Figure 5).

The results in Figure 4 show that the hypocotyl segments produced roots polarly on the NAA or IAA-containing media in the presence of AC (Figure 4A,a , 4B,b and 4D). Roots proliferated from the basal ends of the hypocotyl segments. No-polarity of root formation was observed on media supplemented with NAA or IAA without AC (Figure 4A–b, 4B–a, 4C, 4E, and 4F). Roots were observed on both ends (basal and apical) and/or in the middle of the hypocotyl segments.

Results presented in Figure 3D indicate that rooting also

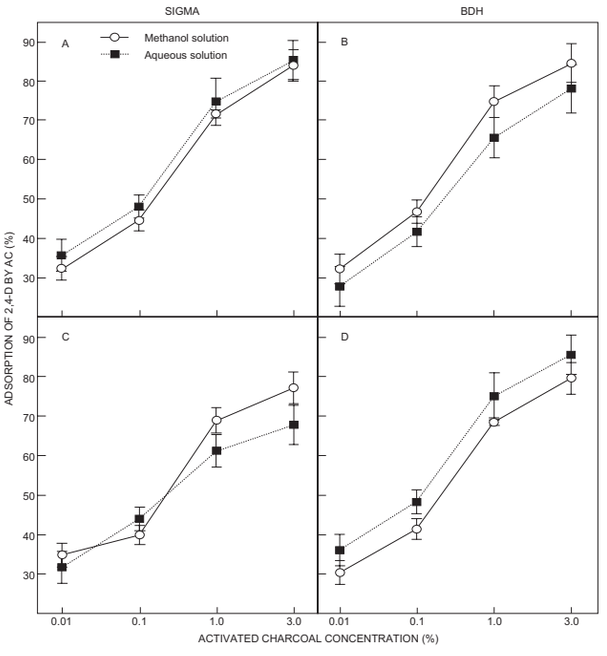

Figure 1: Changes in 2,4-D in methanol and aqueous solutions in the presence or absence of activated charcoal determined by HPLC on the first day after preparation. **A:** 0.5mgml^{-1} 2,4-D added to AC-containing (Sigma) solutions; **B:** 0.5mgml^{-1} 2,4-D added to AC-containing (BDH) solutions; **C:** 1.0mgml^{-1} 2,4-D added to AC-containing (Sigma) solutions; **D:** 1.0mgml^{-1} 2,4-D added to AC-containing (BDH) solutions. Values are the means of three replicates with bars indicating ±SE

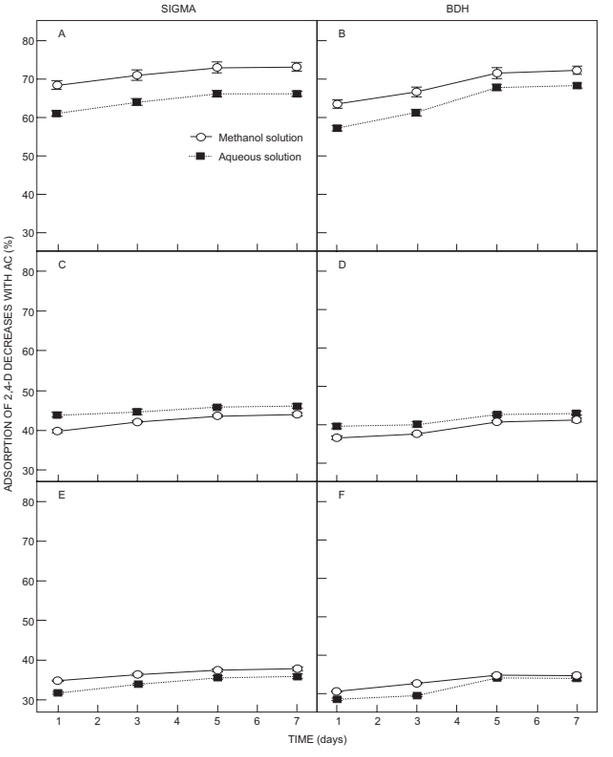

Figure 2: Percentage of 2,4-D (0.5mgml^{-1}) adsorbed by activated charcoal (Sigma and BDH) from methanol and aqueous solutions over seven days. **A:** adsorption of 2,4-D by Sigma AC (0.01%); **B:** adsorption of 2,4-D by BDH AC (0.01%); **C:** adsorption of 2,4-D by Sigma AC (0.1%); **D:** adsorption of 2,4-D by Sigma AC (0.1%); **E:** adsorption of 2,4-D by Sigma AC (1.0%); **F:** adsorption of 2,4-D by BDH AC (1.0%). Values are the means of three replicates with bars indicating ±SE

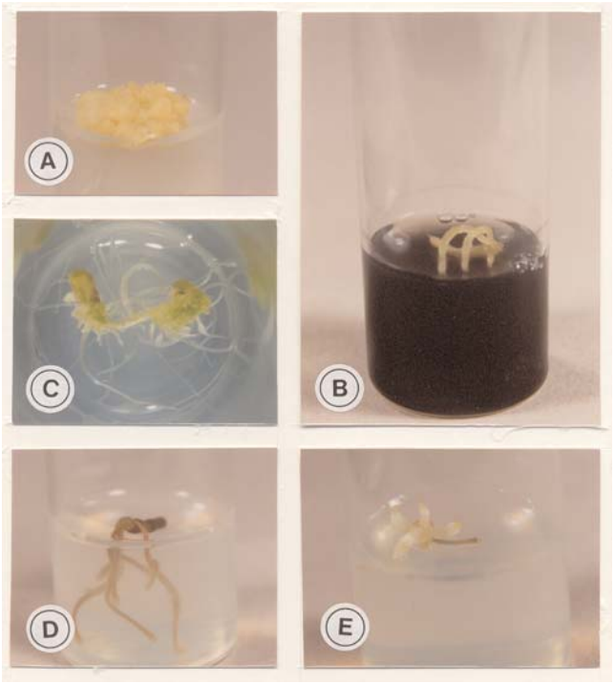

Figure 3: The response of hypocotyl segments of 7-day-old seedlings of *Daucus carota* cultured on MS media supplemented with 1.0mgl^{-1} 2,4-D in the presence or absence of activated charcoal. **A:** callus induced on the medium containing 2,4-D only; **B:** roots induced on the medium containing 2,4-D in the presence of 0.5% AC; **C:** roots induced on the medium containing 2,4-D in the presence of 0.01% AC; **D:** roots induced on the 2,4-D-free medium in the absence of AC; **E:** roots induced on the medium in which AC was removed before autoclaving

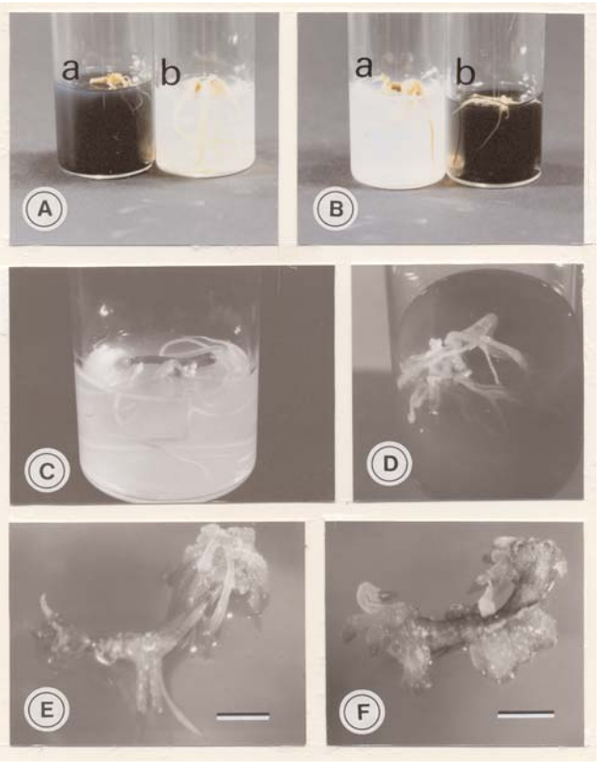

Figure 4: *In vitro* rooting of hypocotyl segments of *Daucus carota.* **A:** on 0.5mgl^{-1} IAA-containing media, a: in the presence of AC; b: in the absence of AC. **B:** on 0.5mgl^{-1} NAA-containing media, a: in the absence of AC; b: in the presence of AC. **C, E:** on 0.5mgl^{-1} IAA-containing media in the absence of AC. Bar = 1mm. **D:** on 0.5mgl^{-1} IAA-containing media in the presence of AC. **F:** on NAA-containing medium in the absence of AC. Bar = 1mm

resulted with the hormone-free media in the presence and/or absence of AC. The rooting percentage of explants and the number of roots per explant on the 2,4-D-free medium in the absence of AC were lower than those on the 2,4-D-containing medium in the presence of AC (Figure 5).

Results presented in Figure 3E show that rooting occurred when AC was removed by filtration prior to media autoclaving [2,4-D (1.0mgl^{-1}) was added to the medium before AC removing]. However, roots were not observed on the medium in which 2,4-D was added after removal of AC from the medium (2,4-D was not added to the medium before AC removal) before autoclaving.

Different responses of hypocotyl segments to various levels of 2,4-D (0mgl^{-1}, 0.5mgl^{-1}, 1.0mgl^{-1}, 3.0mgl^{-1}, 5.0mgl^{-1}, 8.0mgl^{-1} and 10.0mgl^{-1}) were observed on MS media supplemented with 0.02%, 0.1% and 0.5% AC (Table 2). Table 2 indicates that rooting occurred on the media containing 0.5–10.0mgl^{-1} 2,4-D in the presence of 0.1% and 0.5% of AC.

Addition of 0.02% AC to the media containing 0.5–10.0mgl^{-1} 2,4-D resulted in root, shoot and embryo formation (Table 2). Figure 6A shows that addition of 0.02% of AC to the medium containing 3.0mgl^{-1} 2,4-D resulted in root and shoot formation. On the medium containing 5.0mgl^{-1} 2,4-D in the presence of 0.02% of AC, callus and roots were observed (Figure 6B). Embryos and shoots were also induced from the explants (Figure 6C). Rooting also resulted from the hypocotyl segments on the medium containing 8.0mgl^{-1} 2,4-D in the presence of 0.02% of AC (Figure 6D). However, the explants were swollen. Poor callus was observed on the hypocotyls in the medium supplemented with 5.0mgl^{-1} 2,4-D in the absence of AC (Figure 6E).

Root number per explant greatly increased on the IAA or NAA-containing media in the presence (Figures 7A, B and E) or absence (Figures 7C and D) of AC when the explants were pre-cultured on a MS medium containing 1.0mgl^{-1} 2,4-D for 2 to 3 days. Figure 7D also indicates that shoot primordia were induced on the IAA-containing medium in the absence of AC when the explant was pre-cultured on 2,4-D-containing medium for 3 days.

Results presented in Figure 7 show that when hypocotyl segments were pre-cultured on a 2,4-D-containing MS medium for 5 days, embryos emerged from the hypocotyls directly in the presence of AC (Figure 7F). This suggested that longer pre-culturing (5 days) onto 2,4-D leads directly to induction of embryos from explant hypocotyls.

Table 1: Effect of NAA, IAA and activated charcoal on root formation

Treatment			
Auxin (mgl^{-1})	AC (%)	No. of roots per explant	Rooting (%)
NAA 0.1	0	7.3±2.5	84.8
	0.1	4.2±1.8	80.5
	0.5	3.8±1.5	78.0
	1.0	3.1±1.5	62.0
	3.0	2.8±1.0	60.3
NAA 0.5	0	9.8±1.9	86.7
	0.1	4.8±1.7	78.5
	0.5	3.7±1.5	76.2
	1.0	3.0±1.4	72.1
	3.0	2.9±1.1	61.5
IAA 0.1	0	6.7±1.8	84.7
	0.1	4.0±1.7	80.8
	0.5	3.6±1.4	79.5
	1.0	2.4±1.2	66.7
	3.0	2.1±1.0	60.9
IAA 0.5	0	8.9±1.9	85.4
	0.1	5.2±1.8	80.2
	0.5	4.3±1.5	78.5
	1.0	3.1±1.1	68.1
	3.0	2.0±1.0	61.8

Discussion

Addition of AC to a culture medium is a recognised practice in plant tissue culture. The beneficial effects of AC are frequently attributed to its adsorptive properties. It was shown that 2,4-D was effectively adsorbed by AC from methanol and aqueous solutions. Two types (Sigma and BDH) and the four different levels (0.01%, 0.1%, 1.0% and 3.0%) of AC used resulted in reducing the added 2,4-D levels in methanol and aqueous solutions. The adsorptive capacity of AC (Sigma) was greater than the adsorptive capacity of AC (BDH), but the difference was not significant. There was no significant difference in the changes of the level of adsorption of 2,4-D by AC between methanol and aqueous solutions. The highest levels of adsorbed 2,4-D by AC (Sigma and BDH) occurred within the first day. The degree of adsorption of 2,4-D by four AC concentrations (0.01%, 0.1%, 1.0% and 3.0% AC) in both methanol and aqueous solutions slightly increased with time after seven days.

Research workers have shown that the addition of AC often has a promotive effect on the growth and organogenesis of woody species (Martinez-Pulido *et al.* 1990, Dumas and Monteuuis 1995, Sanchez *et al.* 1996). It is possible that AC releases and/or adsorbs substances which promote

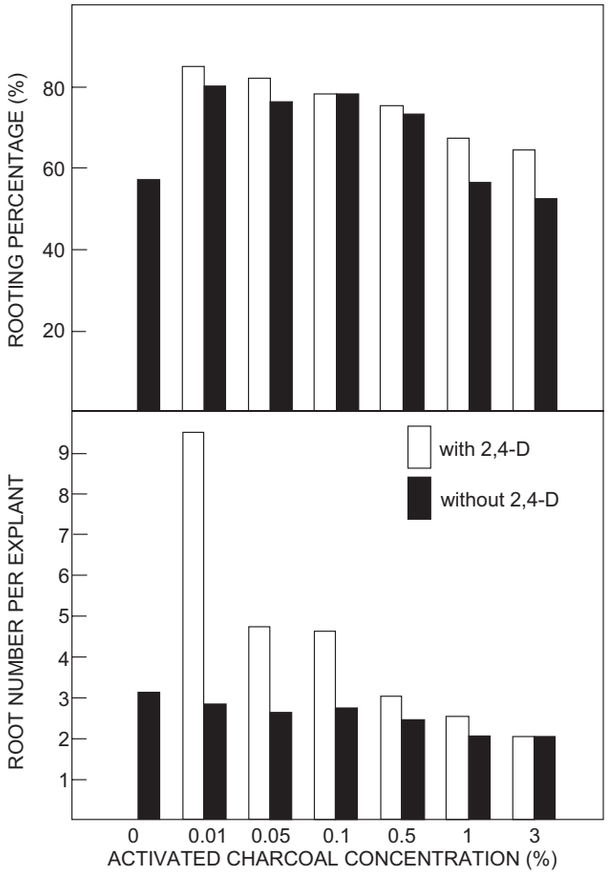

Figure 5: Root number per explant and percentage of hypocotyl segments rooting on MS media with or without $1.0mgl^{-1}$ 2,4-D, in the presence or absence of activated charcoal

and/or inhibit the *in vitro* growth of plants or explants. Although Weatherhead *et al.* (1979) analysed impurities in AC and studied effects of ions of a few metals using anther cultures of *Nicotiana tabacum*, little is known of a possible release from AC of substances that may affect embryogenesis in anther cultures. It cannot be excluded that some of the elements that are not present in MS-medium, but are released in minor amounts from AC, may be beneficial to embryogenesis, as suggested by Weatherhead *et al*. (1979). In that study, impurities in AC was determined by mass spectrometry, and in some case related to embryogenesis. However, the results may not be directly comparable to the

Table 2: The effect of 2,4-D and activated charcoal on the organogenesis by hypocotyl segments of *Daucus carota*

Activated Charcoal(%)	2,4-D Concentration (mgl^{-1})						
	0	0.5	1	3	5	8	10
0	R	C	C	PC	PC	EB	EB
0.02	R	R	R	R/S	C/E/R	R	R
0.1	R	R	R	R	S	R	R
0.5	R	R	R	R	R	R	R

C = Callus, E = Embryos, EB = Explant Browning, PC = Poor Callus, R = Root, S = Shoot

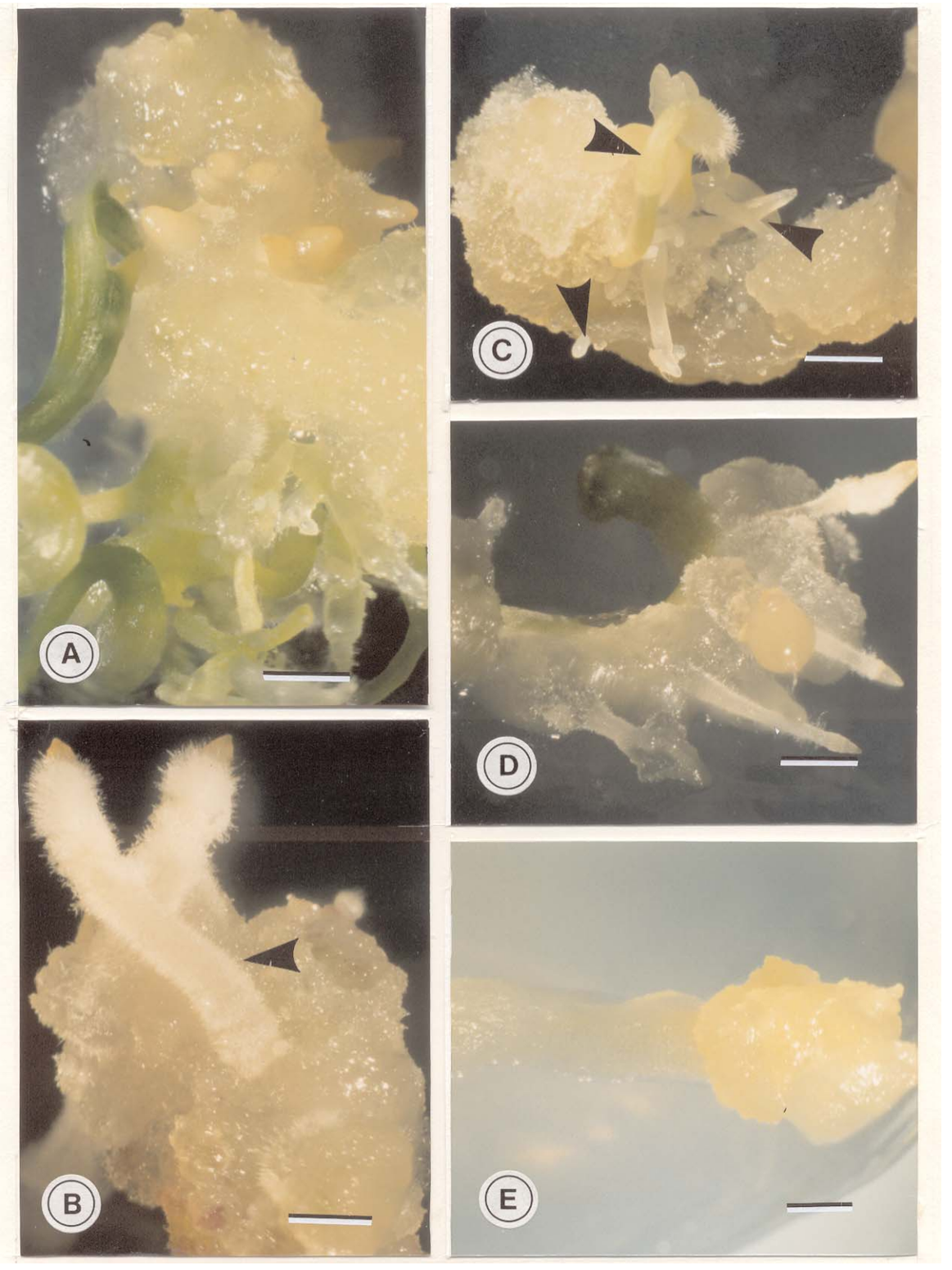

Figure 6: *In vitro* rooting on the hypocotyl segments of 7-day-old seedlings of *Daucus carota* which were cultured on MS media containing 2,4-D in the presence or absence of activated charcoal: **A:** root primordia and shoots on the medium containing 3.0mgl^{-1} 2,4-D in the presence of 0.02% AC; **B:** callus and roots on the medium containing 5.0mgl^{-1} 2,4-D in the presence of 0.02% AC; **C:** embryos and shoots induced on the medium containing 5.0mgl^{-1} 2,4-D in the presence of 0.02% AC; **D:** roots induced on the medium containing 8.0mgl^{-1} 2,4-D in the presence of 0.02% AC; **E:** swollen explant on the medium containing 5.0mgl^{-1} 2,4-D in the absence of AC. Bar = 500µM

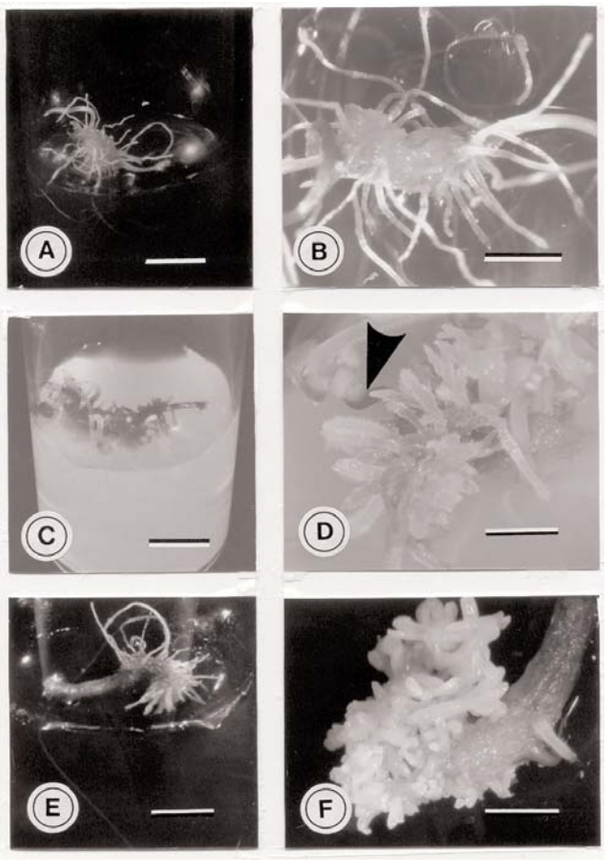

Figure 7: *In vitro* rooting on hypocotyl segments of *Daucus carota* pre-cultured on a MS medium containing 2,4-D for 2 to 5 days. **A:** on the IAA-containing medium in the presence of AC. Bar = 5mm. **B:** amplification of A. Bar = 1mm. **C:** on the IAA-containing medium in the absence of AC. Bar = 5mm. **D:** amplification of C showing shoot primordia as indicated by arrow. Bar = 1mm. **E:** on the NAA-containing medium in the presence of AC. Bar = 5mm. **F:** on the 2,4-D containing medium in the presence of AC. Bar = 1mm

present results as no statement was made as to the release of the impurities into culture media. The brand of AC was also not mentioned.

Activated charcoal has been used in plant tissue culture media to improve growth and/or promote morphogenesis in a wide variety of plant species (George 1993, Pan and Van Staden 1998). In this study, rooting occurred in the MS medium supplemented with 2,4-D in the presence of AC. Roots were not observed on the 2,4-D-containing medium in the absence of AC. Rooting also occurred with the medium containing high concentrations (0.5–8.0mgl^{-1}) of 2,4-D in the presence of 0.02% AC. The beneficial effect of AC on root production is mainly, but may not entirely, be due to the adsorption of 2,4-D by AC. This suggests that AC can adsorb comparatively high concentrations of growth regulators and make them unavailable to tissue explants. In agreement with this, Nissen and Sutter (1990) recommend that 0–100 times more auxins should be added to a medium if high concentrations of AC are used.

There are few reports that 2,4-D can be used for root formation (Boyes and Sink 1981, Shipton and Jackes 1986, Berthon *et al*. 1991). Pre-culturing explants on a 2,4-D-containing MS medium for 2–3 days resulted in an increased root number on the auxin-containing medium in the presence or absence of AC. Generally four phases can be distinguished in the rooting process (George 1996): (a) an induction phase, when the capacity for root formation is determined; (b) an initiation phase, when visible cytological changes occur; (c) an organisation phase, when root primodia can be seen to be produced histologically; and (d) a growth (root elongation) phase when primordia develop into roots. During the root induction phase on rooting induction medium, total uptake of (^{14}C) 2,4-D was higher in the easy-to-root juvenile clone, and was characterised by greater accumulation of radioactivity in the apical part compared to the difficult-to-root mature clone (Berthon *et al.* 1991). It seems that both 2,4-D pre-culturing and AC are directly or indirectly involved in root induction or initiation. It is unknown at which phase AC may exert its effects in the rooting process, and whether rooting might be stimulated by pre-culturing explants on the 2,4-D-containing MS medium for 2 to 3 days. This requires further investigation.

The present study showed that *in vitro* rooting on hypocotyl segments of carrot occurred on MS medium supplemented with IAA or NAA in the presence or absence of AC. Smulders *et al.* (1988) found that addition of 1-naphthaleneacetic acid (1-NAA) led to flower bud development on explants from flower stalks of tobacco cv., Samsun cultured *in vitro*. At low concentration of 1-NAA, bud emerged mainly at the basal edge, whereas at high concentration they developed on the remaining surface. The hypocotyls of *Daucus carota* produced roots polarly on the NAA or IAA-containing media in the presence of AC. The distribution of the roots over the explants was shown to be caused by lowering the NAA or IAA concentration in the NAA and IAA-containing media in the presence of AC. However, no-polarity of root formation was observed on media supplemented with NAA or IAA in the absence of AC. This suggests that AC might affect auxin transport.

Alteration of medium pH, to an optimum level for morphogenesis, has been reported as a beneficial effect of AC (Owen *et al.* 1991). Liu *et al.* (1993) and Blakely *et al*. (1986) suggested that an increase in the acidity of the cell wall pH might facilitate auxin transport across membranes to the site of lateral root primordia formation. An acidic pH increases carrier-mediated uptake of IAA and might allow auxin accumulation at the target sites responsible for rooting. Low pH increased the number of adventitious roots formed by the hypocotyls of sunflower seedlings and acidic conditions may in part promote root formation by increasing the movement of IAA to the rooting zone (Liu *et al.* 1993). The presence of AC to media resulted in a decrease in medium pH (Pan and Van Staden 1999). It is possible that rooting could be enhanced by the acidic pH provided by using AC. The pH of the medium dropped and may be accompanied by a decrease in sucrose with an corresponding increase in the glucose and fructose levels (Pan and Van Staden 1999).

Activated charcoal can provide a dark environment, and if sufficient AC is added to the medium the amount of light

passing through a solidified medium is reduced and/or light can be kept away from the rooting zone. This could promote some physiological reactions which occur in the dark. It is generally thought that auxins are metabolised less rapidly in the dark than in the light (Norton and Boe 1982). Therefore, darkness is generally beneficial to rooting, especially during the inductive phase.

Addition of 0.01 to 0.5% AC to culture media enhanced rooting ability. It is probably due to the fact that AC is also able to adsorb inhibitory substances which might be produced by cultures or be present in the medium. Activated charcoal adsorbed substances, such as products of sucrose breakdown, produced by autoclaving (Weatherhead *et al.* 1978), and/or phenolic compounds which are released from culture cells (Johansson 1983). Addition of 0.01% AC to the culture medium resulted in significantly increases in the root number per explant. A lower root number per explant was obtained by addition of a high concentration of AC to the medium. Activated charcoal is known to adsorb a number of compounds which are normally incorporated in the culture medium such as auxins, cytokinins, abscisic acid (Johansson *et al.* 1982), vitamins (Weatherhead *et al.* 1979), and iron chelates (Heberle-Bors 1980). It is possible that the addition of high concentrations of AC may induce nutrient deficiencies in culture media. Such a deficiency will affect explant growth.

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References

Berthon JY, Boyer N, Gaspar T (1991) Uptake, distribution and metabolism of 2,4-dichlorophenoxyacetic acid in shoots of juvenile and mature clones of *Sequoiadendron giganteum* in relation to rooting *in vitro*. ***Plant Physiology and Biochemistry*** **29**: 355–362

Blakely LM, Blakely RM, Galloway CM (1986) Effects of dimethyl sulfoxide and pH on indoleacetic acid-induced lateral root formation in the radish seedling root. ***Plant Physiology*** **80**: 790–791

Boyes CJ, Sink KC (1981) Morphogenetic responses of *Salpiglossis sinuata* leaf explant and callus. ***Scientia Horticulturae*** **15**: 53–60

Dumas E, Monteuuis O (1995) *In vitro* rooting of micropropagated shoots from juvenile and mature *Pinus pinaster* explants: Influence of activated charcoal. ***Plant Cell, Tissue and Organ Culture*** **40**: 231–235

Ebert A, Taylor HF (1990) Assessment of the changes of 2,4-dichlorophenoxyacetic acid concentrations in plant tissue culture media in the presence of activated charcoal. ***Plant Cell, Tissue and Organ Culture*** **20**: 165–172

Fridborg G, Eriksson T (1975) Effects of activated charcoal on growth and morphogenesis in cell cultures. ***Physiologia Plantarum*** **34**: 306–308

George EF (1993) Rooting and establishment. In: George EF (ed) Plant Propagation by Tissue Culture Part I in Practice 2nd. Exegetic Ltd, Wilts, UK

George EF (1996) Rooting and establishment. In: George EF (ed) Plant Propagation by Tissue Culture Part II in Practice 2nd. Exegetic Ltd, Wilts, UK

Halhouli KA, Darwish NA, Aldhoon NM (1995) Effects of pH and inorganic salts on the adsorption of phenol from aqueous systems on activated decoloring charcoal. ***Separ Science and Technology*** **30**: 3313–3324

Heberle-Bors E (1980) Interaction of activated charcoal and iron chelates in anther culture of *Nicotiana* and *Atropa belladonna*. ***Zeitschrift für Pflanzenphysiologie*** **99**: 339–347

Johansson L (1983) Effects of activated charcoal in anther cultures. ***Physiologia Plantarum*** **59**: 397–403

Johansson L, Andersson B, Eriksson T (1982) Improvement of anther culture technique: activated charcoal bound in agar medium in combination with liquid medium and elevated CO_2 concentration. ***Physiologia Plantarum*** **54**: 24–30

Liu JH, Mukherjee I, Reid DM (1993) Stimulation of adventitious rooting in sunflower (*Helianthus annuus*) by low pH: possible role of auxin. ***Canadian Journal of Botany*** **71**: 1645–1650

Martinez-Pulido C, Harry IS, Thorpe TA (1990) *In vitro* regeneration of plantlets of Canary Island pine (*Pinus canariensis*). ***Canadian Journal of Forest Research*** **20**: 1200–1211

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. ***Physiologia Plantarum*** **15**: 473–497

Nissen SJ, Sutter EG (1990) Stability of IAA and IBA in nutrient medium to several tissue culture procedures. ***HortScience*** **25**: 800–802

Norton ME, Boe AA (1982) *In vitro* propagation of ornamental Rosaceous plant. ***HortScience*** **17**: 190–191

Owen HR, Wengerd D, Miller R (1991) Culture medium pH is influenced by basal medium, carbohydrate source, gelling agent, activated charcoal and medium storage method. ***Plant Cell Reports*** **10**: 583–586

Pan M, Van Staden J (1998) The use of charcoal in *in vitro* culture — A review. ***Plant Growth Regulation*** **26**: 155–163

Pan M, Van Staden J (1999) Effect of activated charcoal, autoclaving and culture media on sucrose hydrolysis. ***Plant Growth Regulation*** **29**: 135–141

Pierik RLM (ed) (1987) *In Vitro* Culture of Higher Plants. Martinus Nijhoff Publishers, Dordrecht

Sanchez MC, San-Jose MC, Ballester A, Vieitez AM (1996) Requirements for *in vitro* rooting of *Quercus robus* and *Q. rubra* shoots derived from mature trees. ***Tree Physiology*** **16**: 673–680

Shipton WA, Jackes BR (1986) Clonal propagation of *Leptospermum* spp. by tissue culture. Plant Cell Reports **5**: 5–8

Smulders MJ, Croes AF, Wullems, GJ (1988) Polar transport of 1-naphthaleneacetic acid determines the distribution of flower buds on explants of tobacco. ***Plant Physiology*** **88**: 752–756

Sriskandarajah S, Mullins MG (1981) Micropropagation of Granny apple: factors affecting root formation *in vitro*. ***Journal of Horticultural Science Letters*** **24**: 1–9

Weatherhead MA, Burdon J, Henshaw GG (1978) Some effects of activated charcoal as an additive to plant tissue culture media. ***Zeitschrift für Pflanzenphysiologie*** **89**: 141–147

Weatherhead MA, Bourdon J, Henshan GG (1979) Effect of activated charcoal as an additive plant tissue culture media. ***Zeitschrift für Pflanzenphysiologie*** **94**: 399–406

Yam TY, Ernst R, Arditti J, Nair H, Weatherhead MA (1990) Charcoal in orchid seed germination and tissue culture media: a review. ***Lindleyana*** **5**: 256–265

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